Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4396–4400, May 1991
Neurobiology

Fast oscillations (20–40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat

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Communicated by R. R. Llinás, March 7, 1991

ABSTRACT **Previous investigations in various motor and sensory cortical areas have shown that fast oscillations (20–80 Hz) of focal electroencephalogram and multiunit activities occur spontaneously during increased alertness or are dependent upon optimal sensory stimuli. We now report the presence of 20- to 40-Hz rhythmic activities in intracellularly recorded thalamocortical cells of the cat. In some neurons, subthreshold oscillations were triggered by depolarizing pulses and eventually gave rise to action potentials. In other neurons, the oscillations consisted of fast prepotentials, occasionally generating full spikes that arose from the resting or even from hyperpolarized membrane potential levels, and leading to trains of spikes at more depolarized levels. The rhythmic nature of these fast prepotentials was confirmed by means of an autocorrelation study, which demonstrated clear peaks at 25-ms intervals (40 Hz). In view of the recent evidence that mesopontine cholinergic nuclei trigger and maintain activation processes in thalamocortical systems, we tested the possibility that stimulation of these brainstem nuclei potentiates the 40-Hz waves on the background of the cortical electroencephalogram. This was indeed the case. The potentiation outlasted the stimulation by 10–20 s. The brainstem-induced facilitation of cortical 40-Hz oscillations was blocked by scopolamine, a muscarinic antagonist. That this facilitation was transmitted by brainstem–thalamic cholinergic projections was confirmed by persistence of the phenomenon after large excitotoxic lesions of the nucleus basalis of Meynert.**

The first demonstration that 40-Hz waves appear on the cortical electroencephalogram (EEG) during an experimental condition mimicking natural arousal was obtained by Bremer *et al.* (1) upon stimulation of the mesencephalic reticular formation (see figure 5 *C–D* in ref. 1). This finding indicated that the EEG counterpart of an increase in the vigilance level does not merely consist of the disruption of synchronized spindles and slow (delta) waves but also includes the appearance of clear-cut oscillations around 40 Hz, with amplitudes exceeding those of background waves. Since then, a series of studies in various motor and sensory cortical areas have reported the presence of spontaneously occurring waves at 35–45 Hz during the behavioral condition of immobility and increased alertness while the animal is watching a prey (2), during accurate performance of a conditioned response (3), and during focused arousal prior to the performance of a complex task (4). In addition, stimulus-dependent oscillations at 25–45 Hz of the focal EEG and/or neuronal firing probability have been described in the olfactory system (5) and visual cortex (6–8). It was also reported that the midbrain reticular stimulation selectively enhances the 80-Hz afterdischarge of the flash-evoked response in the visual cortex (9) and facilitates the coherency of 40- to 45-Hz oscillatory responses of visual cortical cells to appropriate light stimuli (10).

The cellular mechanisms underlying the fast oscillations in thalamocortical loops are not clearly elucidated. Llinás *et al.* (11) have shown that sparsely spinous interneurons recorded *in vitro* from layer 4 of guinea-pig frontal cortex display narrow-frequency (35- to 45-Hz) oscillations upon depolarization of the membrane potential. It was found that these oscillations are generated by a voltage-dependent, persistent sodium conductance, with the involvement of a delayed rectifier.

We report here 20- to 40-Hz rhythmic excitatory events in intracellularly recorded thalamocortical cells of the cat and the potentiation of 40-Hz oscillation in the cortical EEG by brainstem cholinergic projections relayed in the thalamus.

MATERIALS AND METHODS

Experiments were conducted on adult cats anesthetized with urethane (1.8 g/kg of body weight), paralyzed with gallamine triethiodide, and artificially ventilated with control of the end-tidal CO_2 concentration at 3.5 ± 0.2%.

Intracellular recordings were performed with micropipettes filled with a 3.5 M potassium acetate (tip diameter, 0.5 μM; dc resistance, 25–40 MΩ). Thalamic neurons were impaled in the ventroanterior–ventrolateral (VA–VL) complex and the rostral intralaminar centrolateral (CL) nucleus. We analyzed cells with resting membrane potential of at least −55 mV and overshooting action potentials. Conventional criteria were used to ascertain the antidromic invasion of thalamocortical cells from pericruciate gyri. A high-impedance amplifier with active bridge circuitry was used to record and inject current inside the cells. The signals were recorded on tape [pass-band: (dc), 9 kHz] and the recorded data were digitized at a rate of 20,000 samples per second before analysis on a personal computer.

The cortical EEG was recorded from the pericruciate and anterior suprasylvian gyri. Stimulation of the peribrachial area of the pedunculopontine nucleus consisted of three brief (0.1-s) trains of 0.1-ms pulses at 300 Hz or one pulse train (1 s) around 30 Hz. The three short trains at 300 Hz were employed because such a stimulation proved to be most effective in inducing a long-lasting depolarization of thalamocortical neurons (12). The single train at about 30 Hz was used to mimic the discharge rates of peribrachial neurons during brain-activated states in behaving cats (13). Intensities ranged between 0.05 and 0.5 mA.

Scopolamine was administered (0.5–1 mg/kg, i.v.) to block the potentiating effect of brainstem peribrachial stimulation upon cortical 40-Hz waves.

To rule out the possibility that the effect of brainstem stimulation upon the cortical EEG was relayed by the cholinergic aggregates of the basal forebrain, a saline solution containing 1% kainic acid was injected under aseptic conditions in amounts of 0.04–0.06 μl at four different sites of the basal forebrain, covering the nucleus basalis of Meynert and

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Abbreviations: CL, centrolateral; EEG, electroencephalogram; FPP, fast prepotential; VA–VL, ventroanterior–ventrolateral.

diagonal band nuclei (A14.5–A16). The kainic injection was administered under ketamine anesthesia (40 mg/kg, i.m.), and the animals also received benzodiazepine (Valium, 2.5 mg/kg) to prevent the occurrence of epileptic seizures. Recordings began 2–3 days after the injection of kainic acid, to allow the development of histological evidence of the extent of the excitotoxic lesion.

Animals were perfused with 10% formaldehyde under deep pentobarbital anesthesia. The location of stimulating electrodes and the extent of basal forebrain lesions were examined in frontal frozen sections (80 μm) stained with cresyl violet or thionine (see Fig. 4).

RESULTS

Rhythmic (20- to 40-Hz) Excitatory Events in Thalamocortical Cells. Of the 95 intracellularly recorded thalamocortical cells, 23 neurons displayed fast oscillations in response to depolarizing pulses (n = 5) or exhibited spontaneously occurring fast prepotentials (FPPs) (n = 18).

An example of intrinsic oscillation triggered by depolarizing current pulses is shown in Fig. 1. Current pulses of <0.4 nA had no visible effect. However, pulses of 0.4–0.6 nA elicited a subthreshold membrane fluctuation with a frequency of 25–27 Hz (Fig. 1 *A* and *B*). Pulses of higher amplitude (0.8–0.9 nA) induced action potentials arising from some of the depolarizing oscillations (Fig. 1 *C* and *D*).

More often, rhythmically occurring events consisted of FPPs. Antidromically identified thalamocortical cells in the VA–VL and CL nuclei displayed FPPs in the frequency range of 25–45 Hz at the resting membrane potential. The FPPs led to full action potentials at slightly depolarized levels. Both thalamocortical neurons illustrated in Fig. 2 show FPPs,

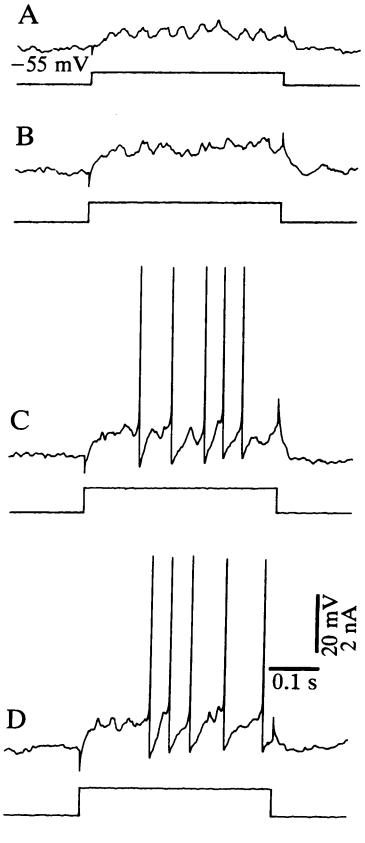

FIG. 1. Subthreshold oscillations at 25–27 Hz elicited by depolarizing pulses in a VL thalamocortical cell. Resting membrane potential is indicated in *A*. The effects of depolarizing pulses with progressively increased amplitudes (0.4, 0.6, 0.8, and 0.9 nA) are shown in *A–D*, respectively.

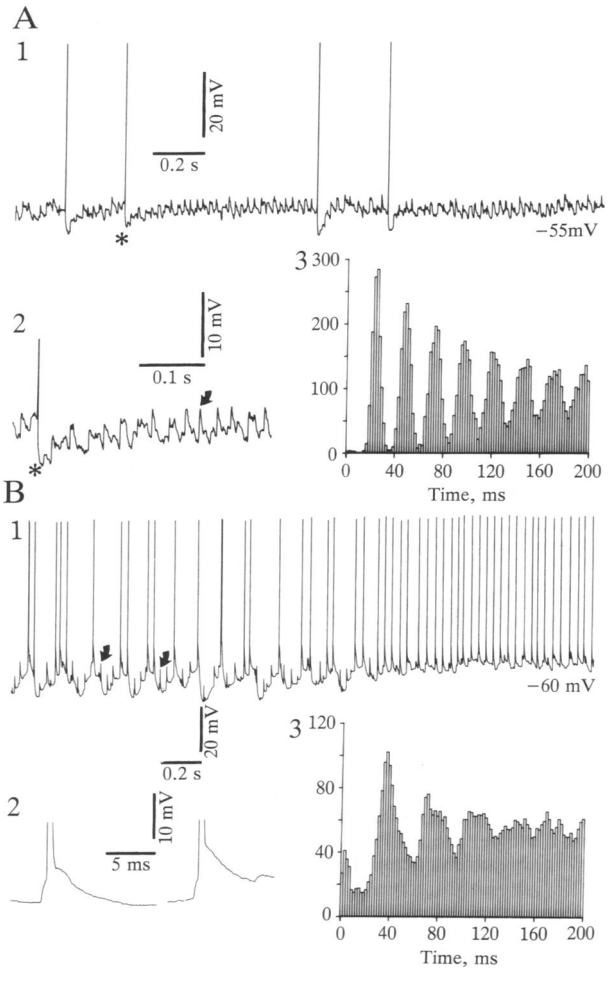

FIG. 2. Fast (30- to 40-Hz), rhythmically recurring prepotentials (FPPs) in two thalamocortical cells. Resting membrane potential is indicated at right in both cells. (*A*) VL cell. An FPP is marked by the arrow in *2* representing the enlarged portion depicted with asterisk in *1* (spike has been truncated in *2*). In *3*, autocorrelogram, computed with a resolution of 2 ms over a time range of 200 ms, indicates multiple peaks recurring with a frequency of about 40 Hz. (*B*) CL cell. Shift from a hyperpolarizing episode to a tonically depolarized epoch is shown in *1*. During membrane hyperpolarization, regularly occurring FPPs (arrows) occasionally boosted full action potentials (see two of them in *2*; spikes have been truncated). In *3*, autocorrelogram, computed from both FPPs and full action potentials, reveals a rhythmicity of about 28 Hz.

sometimes leading to full spikes. The autocorrelogram of the VL cell (Fig. 2*A*) shows multiple peaks, demonstrating an oscillation in the 40-Hz frequency range. The CL neuron (Fig. 2*B*) exhibited FPPs that occasionally boosted full spikes during a period of hyperpolarization, and displayed regularly recurring full action potentials during an episode with spontaneous depolarization; the autocorrelogram computed from both FPPs and action potentials indicates a rhythmicity of 28 Hz.

In some VA–VL neurons, rhythmic FPPs, recurring at a frequency of 35–45 Hz, had two components at the resting membrane potential; these components were stereotypically separated by 2.5 ms (Fig. 3*A 1* and *2*). The FPPs triggered action potentials when the membrane potential was slightly depolarized (arrow in Fig. 3*A*). Superimpositions of oscillatory FPPs showed that the first component constantly had the same slope and amplitude, whereas the second component was either spiky (Fig. 3*B 1*) or roundish with variable amplitudes (Fig. 3*B 2*). Occasionally, the first component appeared in isolation (arrowheads in Fig. 3*B 3*). The same VL cell was driven by stimulating deep cerebellar nuclei and

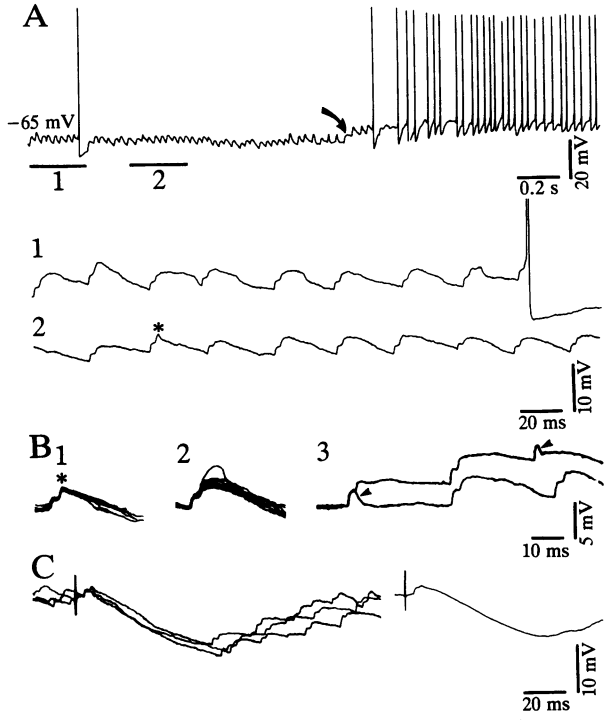

FIG. 3. Rhythmic FPPs in a VL thalamocortical neuron. (*A*) The 35-Hz oscillation of FPPs at the resting membrane potential (−65 mV) and action potentials with the same frequency, produced by application of a steady depolarizing current (arrow). Parts indicated by bars 1 and 2 are depicted below, with increased speed and amplitude to show the biphid character of oscillatory FPPs. Spike was truncated in *1*. (*B*) Superimpositions of 10 oscillatory, biphid FPPs: the first component was stereotyped, whereas the second component was either spiky (asterisk in *1*; see a similar FPP, marked by asterisk in *A 2*) or roundish, with variable amplitudes (*2*); arrowheads in *3* point to the first component in isolation. (*C*) The same cell was driven by stimulating deep cerebellar nuclei (3 superimposed traces at left; 14 averaged traces at right). During cerebellar stimulation, frequency of spontaneous FPPs increased to 45–50 Hz.

displayed evoked FPPs, with the same biphid appearance as the spontaneously occurring ones (Fig. 3*C*). The spontaneous FPPs were completely suppressed during the first phase of the cerebellar-evoked hyperpolarization that followed the early excitation.

Potentiation of 40-Hz Cortical EEG Waves by Stimulation of Mesopontine Cholinergic Nuclei. The origin of rhythmic FPPs recorded in thalamocortical neurons (see Figs. 2 and 3) may be intrinsic. Alternatively, at least some of these rhythmic excitatory events may be extrinsic, arising in projections from the cerebral cortex or brainstem. Since mesopontine cholinergic neurons with identified thalamic projections are known to enhance the excitability of thalamocortical neurons during brain-activated behavioral states (13), we examined the possibility that the 30- to 40-Hz oscillation observed in the cortical EEG was potentiated by mesopontine cholinergic nuclei projecting to the thalamus (14, 15). Brainstem cholinergic nuclei influence the cerebral cortex through two parallel pathways, one relayed through the thalamus and the other through the basal forebrain (16). Because this research focused on the role of the brainstem–thalamic modulatory system, our experiments were carried out after large excitotoxic lesions of the nucleus basalis and diagonal band nuclei. The lesions depicted in Fig. 4 were ipsilateral to the peribrachial stimulating electrode and EEG recordings. In addition, the corpus callosum was sectioned.

To obtain evidence that an increase in the amplitude of fast EEG rhythms may outlast the stimulation of brainstem cholinergic nuclei, we stimulated the peribrachial area with different frequency parameters (see *Materials and Methods*); three brief (0.1-s) pulse trains at 300 Hz were most effective. Fig. 5, depicting the power spectra (0–75 Hz) of cortical EEGs, demonstrates that peribrachial stimulation (at time 0) induced a 2-fold increase of waves around 40 Hz. It also shows that scopolamine abolished the background 40-Hz waves as well as the peribrachial effect.

DISCUSSION

The present results show the presence of 25- to 40-Hz oscillations in thalamocortical neurons and the facilitation of

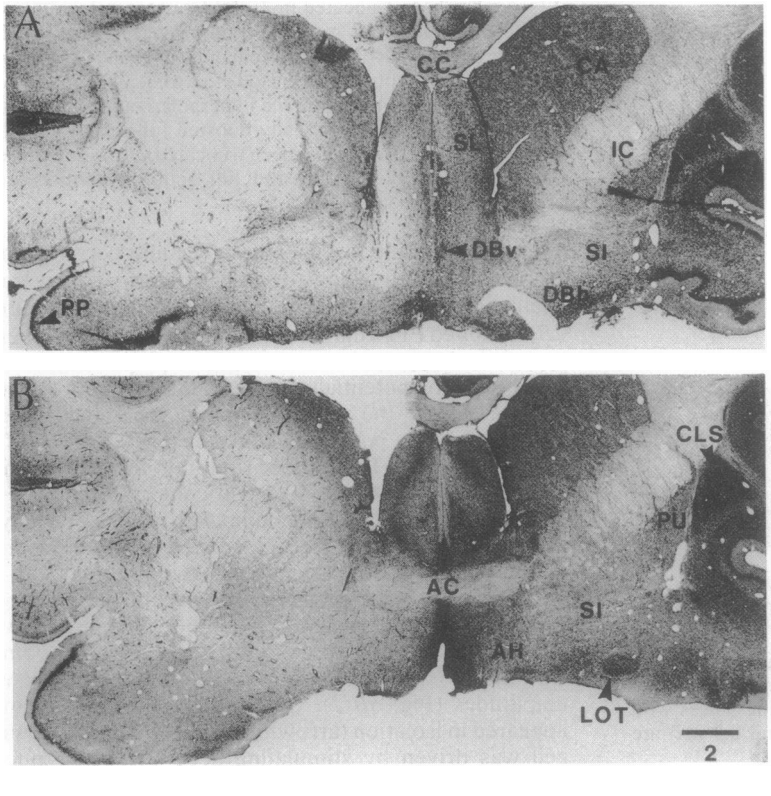

FIG. 4. Excitotoxic lesion of the left basal forebrain by kainic acid injections. *A* and *B* show two frontal sections at A 16.5 and A 14.5. Abbreviations: AC, anterior commissure; AH, anterior hypothalamus; CA, caudate nucleus; CC, corpus callosum; CLS, claustrum; DBh and DBv, horizontal and vertical branches of the diagonal band nuclei; IC, internal capsule; LOT, nucleus of the lateral olfactory tract; PP, prepyriform cortex; PU, putamen; SI, substantia innominata; SL, lateral septum. (Bar = 2 mm.)

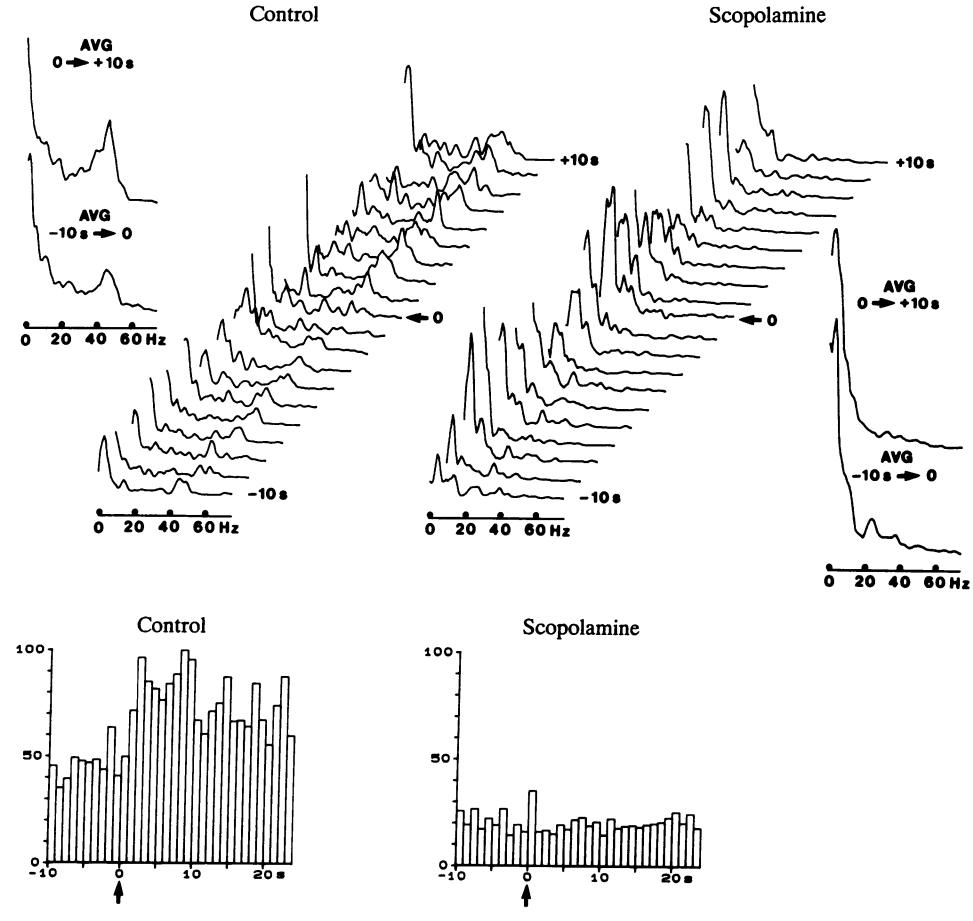

FIG. 5. Potentiation of 40-Hz cortical EEG waves by stimulation of brainstem cholinergic peribrachial area and abolition of this fast rhythm by scopolamine. Epidural implanted electrodes were over the suprasylvian gyrus. (*Upper*) Evolutive power spectra from 20-s epochs, during a control period and after scopolamine (0.5 mg/kg, i.v.). Ten seconds are depicted before (−10 s to 0) and 10 sec after (0 to +10 s) stimulation of peribrachial area (arrow) with three brief (0.1-s) pulse trains at 300 Hz. Averaged (AVG) power spectra are depicted before (−10 s → 0) and after (0 → +10 s) peribrachial stimulation. Note enhancement of 40-Hz waves after peribrachial stimulation in the control condition and abolition of 40-Hz waves after administration of scopolamine. (*Lower*) Histograms of amplitudes of waves between 35 and 45 Hz, before (−10 s to 0) and 24 sec after peribrachial stimulation (delivered at time 0), in the control condition and after administration of scopolamine.

their transmission to the neocortex by brainstem cholinergic systems acting on muscarinic receptors probably located on thalamic cells. In parallel experiments, we have found that thalamic-projecting brainstem cholinergic neurons have a very regular discharge in the frequency range of 25–35 Hz (13).

All these data by no means imply that the fast oscillations are an exclusive property of thalamocortical neurons and that the modulatory system arising in mesopontine cholinergic nuclei is their only potentiating factor. In fact, previous studies have described similar rhythms in the olfactory system, within circumscribed foci of visual cortical areas, or as spontaneous events in various sensory and motor cortical areas during states of focused alertness (see Introduction). The occurrence of 25- to 40-Hz oscillations in so many distant and functionally different neural structures indicates that, in addition to serving as a resonant mechanism for reciprocally coupled groups of cells in separate columns of visual cortex (6, 8, 10), fast rhythms also originate in widespread brain territories and reflect a condition of increased vigilance.

On the basis of our data, we suggest that a major factor accounting for the increase in the incidence and amplitude of these rhythms is the brainstem–thalamic cholinergic projection facilitating the operations of thalamocortical systems. We emphasize that the fast oscillation in thalamocortical neurons may reach the cortex only under conditions that tonically depolarize these elements, thus allowing them to fire action potentials (see Figs. 2*B* and 3*A*). Such a condition is best realized by setting into action the arousing cholinergic cell aggregates at the mesopontine junction (see Fig. 5).

Until now, the presence of fast oscillations in thalamic cells has been a controversial issue because of the failure to find such rhythms in the thalamus. That such a rhythmicity indeed occurs in intracellularly recorded identified thalamocortical cells (see Figs. 1–3) supports a recent hypothesis proposing that the thalamus allows conjunction, by synchronized resonance, between different cortical regions (17). The fact that 25- to 40-Hz oscillations are described here in neurons of the intralaminar CL nucleus is significant in that this nucleus has access to widespread cortical territories (18, 19), including the primary visual cortex (20). The enhancement, by brainstem reticular stimulation, of 40- to 80-Hz light-evoked oscillatory waves in the visual cortex (9, 10) is supported by the present data, which, in addition, showed the cholinergic nature of the phenomenon. The underlying network comprises the brainstem-elicited excitation of cortical-projecting intralaminar thalamic neurons (21).

The problem of the synchronizing device(s) subserving long periods of fast oscillations remains open. Synchronization of many cells is necessary to generate summated events that can be recorded with quite gross electrodes inserted into the brain or even placed on the scalp. Data obtained in slices of rat somatosensory cortex have shown that rhythmic synchronized activity, with a mean frequency of 37 Hz, is displayed by networks of intrinsically bursting, pyramidal-shaped neurons, in a preparation with preserved, while

slightly reduced, inhibition (22). The short duration of oscillations indicated, however, that a limited cortical circuit has a propensity to oscillate but is probably inadequate to sustain oscillations for long periods, as is the case with the present and previous (2) results. One of the possible mechanisms governing the sustained interactions between cell groups is the reentrant signaling (23) that has been used in a set of computer simulations to demonstrate the coherency of oscillatory activity (24).

In addition to corticocortical circuits, a good candidate for the process of synchronization is the thalamocorticothalamic loop. Indeed, the corticothalamocortical circuit does not only return to the same cortical focus but also returns to distant cortical areas. This is the case, for example, of the corticothalamic axons arising in the primary visual cortex and projecting to the lateroposterior nucleus that, in turn, projects to the medial part of the lateral suprasylvian area (25). Of course, such thalamocortical synchronizing circuits would be best realized through the reticular thalamic complex that has widespread projections to most thalamic nuclei (26). The hypothesis was proposed (11, 17) that corticothalamic volleys at 40 Hz impinge upon reticular thalamic cells, thus leading to a 40-Hz inhibitory postsynaptic potential (IPSP)-rebound sequence in thalamocortical neurons which reenters the cortex. During the waking state, when 40-Hz rhythms are best seen and when thalamocortical cells discharge tonically because of their sustained depolarization (19), the inhibitory input from the reticular thalamic complex would sculpture the tonic discharges. Then rhythmic spike trains would be transmitted to the cortex. It is also possible that the resonant thalamocorticothalamic circuits are based on direct excitatory projections, requiring just one interposed synapse in cortical layer VI. This is supported by the recent demonstration of a direct built-in frequency amplification in the corticothalamic pathway. It has indeed been shown that, at 30–50 Hz, cortical stimulation leads to a dramatic increase of excitatory postsynaptic potentials (EPSPs) in directly related thalamic cells (27).

This research was supported by the Medical Research Council of Canada (Grant MT-3689). R.C.D. is a postdoctoral fellow on leave of absence from the University of Padova (Italy). D.P. was a doctoral student in this laboratory and is now working as a Medical Research Council postdoctoral fellow in the Department of Physiology and Biophysics, New York University, School of Medicine.

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